Title of the thesis

ANALYTICAL STUDIES ON SOME WIDELY USED PESTICIDES IN VARIOUS ENVIRONMENTAL SAMPLES

INTRODUCTION

Chhattisgarh is predominantly a tribal state where about 85 percent of the population depends upon agriculture with rice as the main crop. Agricultural and related activities release vast amount of pesticides and other toxicants. Pesticides find their way into the body tissue directly or indirectly through food chain and some of them are quite stable or have unusually long half life, further certain toxic chemicals are neither fully metabolized nor quickly detoxicated and therefore create serious problem or residue accumulation and even death. Organic toxicants like pesticides and related compounds encompass the variety of chemicals to kill or repel unwanted organisms such as insects, animals, weeds and microorganisms from infesting lawns, gardens and crops (1).Toxicants like pesticides are a group of environmental pollutants that pose a significant risk to human health. Due to their constant use and chemical stability, these toxic pollutants are wide spread and may persist as contaminants in the ambient environment (2).

The organic toxicants are highly publicized class of environmental pollutants. Unlike other pollutants whose domain are restricted, pesticides spread to every part of the environment; soil, sediments, rivers, lakes, ponds, groundwater, ocean and even air. Certain pesticides have the ability to persist for a long time in the environment (3).

Pesticides are widely used in agriculture to increase the yield, improve the quality, and extend to storage life of food crop. The pesticide must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal used in specified application. The applied chemicals and their degradation product may be remaining as residues in the agricultural products, which become a concern for human exposure(4).

They are chemical substances used to kill animals, plants, insects and pest in agricultural domestic and industrial settings. The main groups of commonly used pesticides include herbicides, insecticides, fungicides, fumigants and rodenticides. Most of the pesticide are broad spectrum and kill both the target and non-target organisms (5).

With the world population predicted to rise from 6 billion to over 9 billion by 2050, population growth is a driver of increased demand for agricultural products. Pesticides are used in agriculture mainly for the purpose of increasing plant productivity. Synthetic organic insecticides, fungicides and

herbicides are agrochemicals designed to combat the attacks of various pests on crops, However, in many cases hazardous residue are left, becoming a permanent danger to quality of food, environment and consumers health (6).

A number of pesticides are highly toxic and even in very small quantities these pesticides can result in the death of humans and animals, while exposure to a sufficient amount of almost any pesticide can initiate long-term illness. Long-term health problem such as respiratory, memory disorders, dermatological conditions, cancer, depression, neurological deficiencies, miscarriages and breath defects have been known to be associated with pesticide exposure. Depending upon the deviation of exposure of pesticides, short-term adverse acute & chronic health defect can occur. Acute health effects include stinging eyes, rashes, blisters, blindness, nausea, dizziness, diarrhea and death, whereas chronic health effects include infertility, developmental toxicity, immune toxicity and disruption of the endocrine system(7).

CONSUMPTION OF PESTICIDE IN INDIA

In India, agriculture is the backbone of the economy. Nearly 65% of the workforce derives livelihood from agriculture and are therefore exposed to chemical pesticides. India is the fourth largest pesticide producer in the world after the U.S., Japan and China. Domestic consumption is around 39 thousand metric tons (TMT). The production of Indian pesticide industry has almost remained stable at 82000-85000 metric tons (M.T.) during financial year 2010(8).

CONSUMPTION OF PESTICIDE IN CHHATTISGARH STATE

The annual consumption of pesticides in Chhattisgarh state for year 2003-04, year 2004-05, year 2005-06, year 2006-07, year 2007-08, year 2008-09, year 2009-10, year 2010-11, 2011-12, and 2012-13 are 377.36 metric tons (M.T.), 415.88 M.T., 449.30 M.T., 487.05 M.T., 400.00 M.T. 410.00 M.T., 570.00 M.T., 600.00 M.T. and 800 M.T. respectively demand 2013-14 is 1000 M.T. The annual consumption of pesticides respectively increased in Chhattisgarh State (9).

OBJECTIVES:

- Development of new sensitive, selective, cheaper and refine spectrophotometric method for the detection and determination of various widely used pesticides such as isoprothiolane, deltamethrin, profenofos, fenvalerate, chlordane, buprofezin, diquat, fenoxycarb and ethion in various biological and environmental sample.
- Sample is to be prepared by extraction of pesticide using an efficient and selective solvent followed by clean-up procedures to remove interfering species.
- Determination of the level of pesticide residue qualitatively and quantitatively with their breakdown product in the clean-up extract using simple sensitive and selective spectrophotometric method will be down as an alternative of costly sophisticated instrumental techniques.

A Brief Review of the work already done

Various methods are used and many techniques have been developed for analyzing detection and determination of pesticides include Liquid Chromatography-Mass Spectrometry(LC/MS)[10], Gas Chromatography with Electron-Capture Detection[11], Gas Chromatography-Mass Spectrometry Determination[12], Reversed-Phase Liquid Chromatography-Gas Chromatography using the Through-Oven Transfer Adsorption/Desorption Interface[13], Two-Dimensional Capillary Gas Chromatography-Time-of-Flight Mass Spectrometry[14], Microwave Accelerated Selective Soxhlet Extraction with Gas Chromatography/Mass-Spectrometry[15], Biosensor Detection[16], Microwave Assisted Extraction and GC-FPD Analysis[17], Liquid Chromatography/Electro spray Ionization tandem Mass-Spectrometry(LC/ESI-MS) and Ultra High Performance Liquid Chromatography Quadrupole Time-of-Flight Mass-Spectrometry(UHPLC QqTOF MS) [18], Gas Chromatography-Ion Trap Mass-Spectrometry[19], Cloud Point Extraction and High-Performance Liquid Chromatography[20] etc. Most of the above mention instrumental methods and techniques are most expensive, quite elaborate, require expensive instruments, maintenance, too sophisticated for field measurement and are difficult to be used in the routine analysis of different matrices.

Noteworthy Contribution in the field of proposed work:

Earlier some work has been already done at school of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh. Simple and sensitive spectrophotometric methods, Thin Layer Chromatography (TLC), Solid Sorbent System and low cost monitoring methods have been developed for the detection and determination of some widely used pesticides in the environment.

A simple spectrophotometric method has been proposed for the determination of widely used pyrethroid pesticides, cypermethrin and fenvalerate. The method is based on the hydrolysis of these pesticides, upon which they release cyanide. The cyanide is reacted with bromine and pyridine to form glutaconic aldehyde, which is then coupled with sulphanilic acid to give an orange red dye in acidic medium having maximum absorbance at 480 nm. Beer's law is obeyed in the ranges of 0.18–1.12 and 0.08–0.64 μg/mL for fenvalerate and cypermethrin, respectively. The proposed method is sensitive and found to be free from interferences of a large number of foreign species and other pesticides. The proposed method could be applied to the determination of fenvalerate and cypermethrin in their formulations and environmental samples (21).

A highly sensitive spectophotometric method is developed for the determination of parts per million levels of widely used organophosphorus pesticide monocrotophos. The method is based on alkaline hydrolysis of monocrotophos to N-methylacetoacetamide followed by coupling with diazotized 2, 4- dinitrophenyl hydrazene in alkaline medium. The absorption maxima of the yellow coloured compound formed is measured at 490 nm. Beer's law is obeyed over the concentration range of 1.5 to 6.0 μg in a final solution volume of 25 ml. The molar absorptivity and Sandell's sensitivity were found to be $5.1x10^5$ (±100) l $mole^{-1}cm^{-1}$ and 0.008 μg cm^{-2} respectively. The standard deviation and relative standard deviation were found to be ± 0.006 and 2.5% respectively. The method is simple sensitive and free from interferences of other pesticides and diverse ions. The method has been satisfactorily applied to the detection/determination of monocrotophos in environmental, agricultural and biological samples (22).

A new and highly sensitive spectrophotometric method is developed for the determination of sub ppm levels of the widely used organophosphorus insecticide dichlorvos. The method is based on alkaline hydrolysis of dichlorvos to dichloroacetaldehyde followed by coupling with diphenyl semicarbazide (DPC) in alkaline medium. The absorption maxima of the wine red dye compound formed is measured at 490 nm. Beer's law is obeyed over the concentration range of 4.3 to 34 μg in a final solution volume of 25 mL (0.18-1.36 ppm). The molar absorptivity, Sandell's sensitivity and correlation coefficient were found to be 2.9×10^5 l $mole^{-1}cm^{-1}$, 0.013 μg cm^{-2} and 0.9999, respectively. The standard deviation and relative standard deviation were found to be ± 0.007 and 1.90%, respectively. The lower limit of detection is 0.04 μg. The method is simple, sensitive and free from interferences of other pesticides and diverse ions. Other organophosphorous pesticides do not interfere with the proposed method. The method has been satisfactorily applied to the determination of dichlorvos in environmental and agricultural samples (23).

A new and highly sensitive spectrophotometric method was developed for the determination of parts per million levels of widely used cypermethrin insecticide. The method is based on alkaline hydrolysis of cypermethrin to cyanide ion, which further reacts with potassium iodide and leuco crystal violet. The absorption maxima of the crystal violet dye formed was measured at 595 nm in acidic medium. Beer's law obeys over the concentration range of 3.0 to 17 µg in a final solution volume of 25 ml (0.12-0.68 ppm). The molar absorptivity and Sandell's sensitivity were found to be $3.3´10^5$ L mol^{-1} cm^{-1} and 0.054 µg cm^{-2}, respectively. The standard deviation and relative standard deviation were found to be ± 0.001 and 0.22%, respectively. The method is simple, sensitive and free from interferences of other pesticides and diverse ions. Other pyrethroid insecticides do not interfere in the proposed method. The method has been satisfactorily applied to the determination of cypermethrin in environmental and biological samples (24).

A sensitive method has been developed for spectrophotometric determination of an organophosphorous pesticide dichlorvos, also known as 2,2 dichlorovinyl methyl phosphate or dichlorophos. The method is based on hydrolysis of dichlorvos by sodium hydroxide to produce dichloroacetaldehyde, which on coupling with phloroglucinol in alkaline medium, gives orange colour. The orange dye shows absorption maxima at 475 nm and obeys Beer's law in the range of 10 - 100µg/25 mL (0.4 to 4 ppm) of solution. The molar absorptivity and Sandell's sensitivity were found to be 4.53 x 10^4 1 mol^{-1} cm^{-1} and 0.0048 µg cm^{-2} respectively. The method has been successfully applied for the determination of dichlorvos in water, agricultural soil and vegetables (25)

A sensitive, selective, cheaper and extractive spectrophotometeric method has been developed for the detection and determination of Baygon in fruits, vegetables, and grains is based on the coupling of their hydrolysation products with diazotized aniline. The dyes formed are measured at 450 nm for Baygon after extraction in chloroform. Beer’s law is obeyed over concentration ranges of 0.8-5.0µg. The Molar absorptivity and Sandell’s sensitivity were found to be 9.7×10^5 L mol^{-1} cm^{-1} and 0.5×10^{-4} µg cm^{-2} respectively. The standard deviation and relative standard deviation were observed as ± 0.00336 and 0.0145% respectively. Various important analytical parameters were evaluated. The method was applied successfully to the determination of Baygon in water, grain, fruits, plant material and biological sample (26).

An extractive, sensitive spectrophotometer method has been developed for the detection and determination of paraquat using glucose (as easily available reducing agent). Paraquat is reduced with glucose in alkaline medium to give a blue colored ion with an absorbance maxima at 610 nm. Beer’s law is obeyed in the range 0.5-5.0 µg of paraquat in 10ml of the final solution (ppm). The

important analytical parameters and the optimum reaction conditions were evaluated. The method was applied successfully to the determination of paraquat in water, grain, plant material and biological sample (27).

Proposed Methodology:

- Survey of widely used pesticide in working environment of Chhattisgarh.
- Sampling of widely used pesticides from different selected areas of Chhattisgarh will be done.
- Simple Spectrophotometric analytical method will be develop to find out the widely used pesticide concentration level in various environmental sample.
- Development of new sensitive and selective spectrophotometric method for the determination of various widely used pesticide in different matrices.
- Study of statistical parameters viz. standard daviation, Relative standard deviation, Sandell's sensitivity, Molar absorbtivity.
- Optimization of analytical parameters like pH, reagent concentration, range temperature, diverse ion effects and application in various environmental biological samples.

Expected Outcomes of The Proposed Work:

- With the demand to overcome the need of food for population which is multiplying rapidly the use of pesticides also increased.
- The practical and scientific Utility of the research work is to make aware the common people, industrialists, manufacturers to these dreadful substances which are threatening our environment and to identify their levels in different matrices.
- The develop method can be directly applied to fill measurements in our state and will be useful to improve our ability to predict their real need so that the farmers can get economic returns from the proper investment of pesticides.
- Method being low cost can be utilized in small laboratories, helpful for agricultural departments, scientist to take suitable step for controlling the indiscriminate use of pesticides.
- Various environmental samples such as water, soil, fruits, and some biological samples are used for the study of the effected of widely used pesticides in the laboratory.

Table-1: The Chemicals/Compounds are generally used as Pesticides.

S.No.	Chemaical/ Compound Name	Classification	LD_{50} value Mg/kg(rat) Oral/dermal	Toxic Effect
1.	Alpha cypermethrin	Pyrethroids	187 to 326	Affect the central nervous system (brain and spinal cord) causing writhing,salivation and clonic convulsions.
2.	Aldrin	Organochlorine	39-64	Produce termors , hyperexcitadibility, seizures and coma.
3.	Borax	Carbonate	869- 1271	Respiratory and skin irritation, diarrhea, kidney failure.
4.	Bitertanol	Triazole	5000	Effect central nervous system, sedation, spasms, respirator disturbance.
5.	Carbofuran	Carbamate	6-18	Nausea, dizziness, respiratory paralysis, increased blood pressure
6.	Cypermethrine	Pyrethroids	250	Skin and eye irritation, Effect central nervous system, increases liver and kidney weight, carcinogenic.
7.	Deltamethrin	Pyrethroids	67	Paralysis, dermatitis, edema, carcinogenic.
8.	Dimethoate	Organothiophosphate	60-387	Repiratory paraltsis, increased blood pressure, carcinogenic.
9.	Dichloro Diphenyl Trichloro Ethane	Chlorinated hydrocarbons	250	Causea headache, blurred vision, muscular vision and muscular weakness.
10.	Diquat	Ethyl bipyridyl	120	Neurological effect, mouth, throat, infection.
11.	Hexaconazole	Triazole	1453	Carcinogenic, neurotoxic.
12.	Monocrotophos	Organophosphate	23	Inhibit action of cholinesterase, disturpt nervous system
13.	Parathion	Organophosphate	3	Carcinogenic, depression, speech difficulties, sleep walking.
14.	Propoxur	Carbamate	40	Inhibit action of cholinesterase, distrupt nervous system.
15.	Phosphamidon	Organophosphate	9/367	Affect central nervous system, paralysis of body extremities and respiratory muscles.

16.	Bifenthrin	Pyrethroids	70	Ulcerations in mouth, headache, dizziness and fatigue.
17.	Buprofezin	Thiadiazine	>5000	Carcinogenic, skin and eye irritation, allergic.
18.	Isoprothiolane	Fungicide	300-2000	Endocrine disrupter, neurotoxic.
19.	Acetamiprid	Neonicotinoide	571	Carcinogenic, neurotoxic, mutagenic, eye and skin irritation.
20.	Prophenofos	Organophosphate	>2000	Headache, dizziness, nausea, vomiting, abdominal cramps.
21.	Mancozeb	Dithiocarbamates	>5000	Fatigue, headache, carcinogenic and birth defect.
22.	Butachlor	Chloroacetanilide	2620	Carcinogenic and neurotoxic.
23.	Bitertanol	Triazole	5000	Effect central nervous system, sedation, spasms, respiratory disturbance.
24.	Maneb	Carbamate	5000-8000	Goiter, reduce growth rate, gastrointestinal disturbance.
25.	Difenacoum	Rodenticide	1.8-2	Nausea, vomiting, anticoagulaent, poisioning.
26.	Phenothrin	Pyrethriods	>5000	Neurotoxic, carcinogenic, eye irritation.
27.	Cyfluthrin	Pyrethriods	869-1271	Facial skin and eye irritation, inflammation of kidney, carcinogenic.
28.	Fenoxycarb	Carbamate	10,000	Carcinogenic, skin and eye irritation, allergic.
29.	Hexazinone	Triazine	1690	Damage liver and heart, termors.
30.	Cyprodinil	Organonitrogen	>2000	Inhibit action of cholinesterase, distrupt nervous system.

REFERENCES

1. Posecion N., Ostrea E., Bielawski D., Corrion M., Seagraves J., Jin Y.," Detection of Propoxture to Environmental Pesticides During Pregnancy by the analysis of Maternal Hair Using GC-MS",*Chromatographia***, 2006**,64(11-12),681-687.
2. Metayer C., Buffler P.A., "Residential Exposures to pesticides and Childhood Leukemia*",Radition protection Dosimetry*, **2008**, vol.132,No. 2,pp.212-219.
3. Odokuma L.O., Akubuenyi F.C.,"Effect of agriculture pesticides on the degradation of medium spill concentration of bonny light crude oil in a tropical rain forest soil",*African Journal of biotechnology*, **2008**,vol.7(4),pp.459-471.
4. Disika.R., Tangirala.s. and Naishadham.T. "pesticide residue analysis of fruit and vegetables," *Gernal of Evs. Che. and toxi.***,2012**, pp-19-28, 22 **.**
5. Sharma D.R., Thapa. R.B., Shrestha S.M. and Pradhan S.B. **"**used of pesticides in Nepal and impects on Human health and Environment**".** *The Jan. of Agri. And Envir.*, **2012,** *Vol. 13.*
6. Murthy P.K.,Sulochana M., Naidu N.V. " A novel spectroscopic method for the determination of carbaryl in environmental samples**"** *Pelagia Research Library Der Pharmacia Sinica***, 2012,** 224-228,
7. Hussain Z., Siddique S**.** "Determination of pesticides in fruit & Vegetables using Acetonitrile , Extraction and GC/MS technique. *Journal of scientific Research*, **2010,** vol. XXXX No. 2.
8. Indian Pesticides Industry" *Market research Report*, September, **2011.**
9. "Directorate of agriculture",*Indira Gandhi agriculture university*, Raipur(C.G.), Annual report., **2013.**
10. Fillatre, Y., Rondeau, D., Bonnet, B., Daguin, A., Jadas-hecart, A., Communal, P., "Multiresidue Analysis of Multiclass Pesticides in Lavandin Essential Oil by LC/MS/MS Using the Scheduled Selected Reaction Monitoring Mode", *Anal. Chem*. **2011**, 83, 109-117.
11. Hunter, R.E., Riederer, A.M., Ryan, P.B., "Method for the Determination of Organophosphorous and Pyrethroid pesticides in Food via gas Chromatography with Electron-Capture Detection", *J. Agric. Food Chem*. **2010**, 58, 1396-1402.
12. Li, Z., Li, Y., Liu, X., Li, X., Zhou, L., Pan, C., "Multiresidue Analysis of 58 Pesticides in Bean Products by Disposable Pipet Extraction (DPX) Cleanup and Gas Chromatigraphy-Mass Spectroscopy Determination", *J. Agric. Food Chem*. **2012**, 60, 4788-4798.
13. Cortes, J.M., Toledano, R.M., Villen, J., Vazquez, A., "Analysis of Pesticides in Nuts by Online Reversed-Phase Liquid Chromatography Using the Through-Oven Transfer Adsorption/Desorption Interface", *J. Agric. Food Chem*. **2008**, 56, 5544-5549.

14. Hyward, D.G., Pisano, T.S., Wong, J.W., Scudder, R.J., "Multiresidue Method for Pesticides and Persistent Organic Pollutants (POPs) in Milk and Cream Using Comprehensive Two-Dimensional Capillary Gas Chromatography-Time-of-Flight Mass Spectrometry", *J. Agric. Food Chem*. **2010**, 58, 5248-5256.
15. Zhou, T., Xiao, X., Li, G., "Microwave Accelerated Selective Soxhlet Extraction for the Determination of Organophosphorus and Carbamate Pesticides in Ginseng with Gas Chromatography/Mass Spectrometry", *Anal. Chem*. **2012**, 84, 5816-5822.,
16. Roepcke, C.B.S., Muench, S.B., Schulze, H., Bachmann, T., Schmid, R.D., Hauer, B., "Analysis of Phosphorothionate Pesticides Using a Choroperoxidase Pretreatment and Acetylcholinesterase Biosensor Detection", *J. Agric. Food Chem.* **2010**, 58, 8748-8756.
17. Niell, S., Pareja, L., Gonzalez, G., Gonzalez, J., Vryzas, Z., Cesio, M.V., Mourkidon, E.P., Heinzen, H., "Simple Determination of 40 Organophosphate Pesticides in Raw Wool Using Microwave-Assisted Extraction and GC- FPD Analysis", *J. Agric. Food Chem.* **2011,** 59, 7601-7608.
18. Wang, J., Leung, D., Chow, W., "Application of LC/ESI-MS/MS and UHPLC QqTOF MS for the Determination of 148 Pesticides in Berries", *J. Agric. Food Chem.* **2010**, 58, 5904-5925.
19. Karanasios, E., Tsiropoulos, N.G., Kapouzas, D., Ehaliotis C., "Degradation and Adsorption of Pesticides in Compost-Based Biomixtures as Potential Substrates for Biobeds in Southern Europe", *J. Agric. Food Chem.* **2010**, 58, 9147-9156.
20. Savant, H.S., Banerjee, K., Utture, S.C., Patil, S.H., Gupta, S.D., Gaste, M.S., Adsule, P.G., "Multiresidue Analysis of 50 Pesticide in Grape, Pomegranate, and Mango by Gas Chromatography-Ion Trap Mass Spectrometry", *J. Agric. Food Chem.* **2010,** 58, 1447-1454.
21. Tamrakar U., Gupta V. K., and Pillai Ajai K. , "A Spectrophotometric Method for the Determination of Fenvalerate and Cypermethrin in Presence of Each Other." *Journal of Analytical Chemistry*, **2012**, Vol. 67, No. 5, pp. 437–442.
22. Sharma P.K. and Rajput S.K., "A new sensitive and facile Spectrophotometric Determination of Monocrotophos in Environmental, Biological and Agricultural Samples." *Int. Jour. of Sci. & Engi. Res*, **2012**, Vol. 3, Issue 10,
23. Janghel E. K., Rai M. K., Gupta V. K. and Rai J. K.,"Trace Spectrophotometric Determination of Dichlorvos using Diphenyl Semicarbazide (DPC) in Environmental and Agricultural Samples." *Journal of the Chinese Chemical Society*, **2007**, *54*, 345-350.

24. Janghel E. K., Rai J. K., Rai M. K., Gupta V. K., "A new sensitive spectrophotometric determination of cypermethrin insecticide in environmental and biological samples." *J. Braz. Chem. Soc.,* **2007**, vol.18 no.3.

25. Asthana A. Pillai A.Gupta V. K. , "A Simple Sensitive Spectrophotometric Method for Determination of Dichlorvos in Environmental Samples." *Indian National Science Academy* , **2003**, vol. 10, n^{o}1, pp. 96-98.

26. Patel V., Rai M.K., and Rai J.K., "A new spectrophotometric method for the determination of Baygon inenvironment and biological samples." *Recent Research in Science and Technology*, **2013**, 5(2): 01-03.

27. Khatoon R., Rai M.K. and Rai J.K., "Low cost spectro photometric determination of paraquat in environmental and biological sample*." Recent Research in Science and Technology,* **2013**, 5(2): 04-06.

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